Monitoring Nitrite, *N*-Nitrosodiethanolamine, and Mutagenicity in Cutting Fluids Used in the Metal Industry

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We carried out an integrated environmental/biological monitoring program to evaluate cancer hazards among metal industry workers exposed to cutting fluids. Several cutting fluids were sampled according to response to a semiquantitative nitrite rapid test in metal factories in central Italy. The nitrite-positive samples were analyzed for nitrite and nitrosodiethanolamine (NDELA) content and mutagenic activity. The nitrite-negative samples were analyzed only for mutagenicity. Of the total samples, 20.6% were nitrite positive, and all contained NDELA. However, nitrite content was not quantitatively predictive of the NDELA content, which varied enormously among samples (0.3–1900 mg/kg). Nitrite-negative samples were always nonmutagenic. Mutagenicity was found in half the NDELA-containing samples but was not related to nitrite or NDELA content. Nitrite screening of cutting fluids in the field is an interesting method for identifying samples that potentially contain NDELA and other unknown mutagens and, when performed with short-term mutagenicity tests, nitrite screening seems to be a valid tool by which industrial managers and health officers could minimize the health hazards associated with occupational exposure to cutting fluids. **Key words:** cutting fluids, metal industry, mutagenicity, nitrite screening, *N*-nitrosodiethanolamine. *Environ Health Perspect* 101:126–128(1993).

Nitrosable amines and amine derivatives have an important role in many industrial processes. Handling, production, and use of these chemicals may result in exposure to the corresponding *N*-nitroso products, which could have mutagenic or carcinogenic activity (*1*). Cutting fluids are widely used to reduce the temperature of the metal–tool interface during metal cutting or grinding and have been found to contain some *N*-nitrosamines. *N*-nitrosodiethanolamine (NDELA) is the most common *N*-nitrosamine in cutting fluids, which are formulated with ethanolamine and nitrite, the precursors of NDELA (*2*). NDELA is a strong animal carcinogen (*3–6*), a mutagen in the Ames test after activation with alcohol dehydrogenase (*7,8*), and a potent inducer of DNA damage in primary hepatocytes *in vitro* (*7,9*). For these reasons NDELA could represent a health risk for workers in the metal industry, who are exposed by direct skin contact or by inhalation of the oil mist.

Following an integrated environmental/biological monitoring approach previously used by our research group in different working environments (*10*) and drawing from published studies of NDELA monitoring in metal industries (*11–13*), we evaluated exposure of metal workers to NDELA and other potentially genotoxic compounds found in cutting fluids. We analyzed cutting fluids sampled in different metal factories in central Italy for nitrite and NDELA content and mutagenic activity. Biological monitoring was carried out on workers who used cutting fluids that were positive for NDELA by analyzing NDELA content of urine, assessing mutagenicity, determining thioether and D-glucaric levels, and determining sister chromatid exchanges (SCE) in peripheral blood lymphocytes. This paper deals only with the environmental monitoring phase. Biological monitoring data are still being gathered.

We collected new and used cutting fluid samples (N = 63) during working hours in several metal factories, took them to the laboratory, and tested them for nitrite content by a rapid and semiquantitative method. The samples that were positive for nitrite, a NDELA precursor, were analyzed for nitrite content by a quantitative method, NDELA content, and mutagenic activity. Nitrite-negative samples were analyzed only for mutagenicity to detect mutagens other than NDELA. The scheme of the environmental monitoring approach followed is given in Figure 1.

Nitrite detection was first performed using a rapid and semiquantitative screening method (Merckoquant, nitrite test, Merck, Germany), where test strips were immersed in the emulsions. We performed the quantitative nitrite determination only on nitrite-positive samples, using an analytical procedure that is an adaptation of standard nitrite/nitrate determination to a continuous flow analyzer. A 1-ml aliquot was used for the determination of nitrite using a dual-channel, continuous-flow nitrite/nitrate analyzer (*14*). Samples positive for nitrite were combined with 1–2 g/100 ml of NaOH and analyzed for NDELA determination. We treated 0.5 g of cutting fluids with about 2 g sulfamic acid to destroy the nitrite and added distilled water to yield 15 ml. Extraction was done using a silica column containing a layer of ascorbic acid (to trap ethanolamines) and a layer of potassium carbonate (to trap ascorbic acid), eluted with 50 ml ethyl formate containing 2% methanol. We evaporated extracts to dryness under a stream of nitrogen and reacted the residue with 0.3 ml of a silylating agent (*N*-methyl-*N*-trimethylsilylheptafluorobutyramide) at 80°C for 2 hr. NDELA quantification was carried out by gas chromatography/chemiluminescence detection (TEA 502) (*12,13*). The gas chromatograph conditions were: injector, 200°C; on-column injection; column, 0.635 cm o.d., 0.2 cm i.d. × 140 cm silanized boro-silica glass filled with 6% OV275 on Volaspher A2 (Merck, Germany); oven, initial temperature 110°C, 5 min, temperature program 10°C/min, final temperature 220°C, 5 min. We assessed the mutagenic activity of the samples directly, as *in toto* samples, and as basic fractions, presumably containing nitrosamines. The *in toto* samples were filter sterilized, added with Tween 80 and tested at increasing doses (up to 150 µl/plate), with the plate-test version of the Ames test (*15*), according to the Hermann

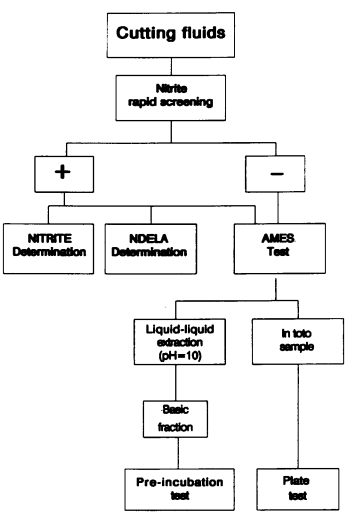

Figure 1. Scheme of the analysis of cutting fluids (N = 63) sampled in Italian metal factories.

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Table 1. Concentrations of nitrite, *N*-nitrosodiethanolamine (NDELA), and mutagenicity in new or used cutting fluids found to contain nitrite by means of a rapid and semiquantitative method

Factory	Sample	New/ used	Nitrite (mg/kg)	NDELA (mg/kg)	Mutagenicity, TA100			
					In toto		Basic fractions	
					–S9	+S9	+S9	+ADH
A	A1	New	13	5.0	–	–	+	–
	A2	Used	55	24.0	–	–	+	–
	A3	Used	2540	1900	–	+	+	+
B	B1	New	2600	17.9	–	–	+	–
	B2	Used	1800	9.1	–	–	+	–
C	C1	New	6500	5.3	–	–	+	–
	C2	Used	5700	6.5	–	–	+	–
D	D1	New	2	0.4	–	–	–	–
	D2	Used	4	0.3	–	–	–	–
E	E1	New	70	2.4	–	–	–	–
	E2	Used	10	0.3	–	–	–	–
F	F1	Used	60	1.0	–	–	–	–
G	G1	New	19,600	30.7	–	–	+	–

See text for details on methods. Mutagenicity results were obtained with the Ames plate test (*15*) for the *in toto* samples and with the preincubation modification of the Ames test (*17*) for the basic fractions, using TA100 strain with either a post-mitochondrial liver fraction obtained from Aroclor-induced rats (+S9 mix), or alcohol dehydrogenase (ADH) activation. These data are expressed only qualitatively (see Table 2 for dose–response results; the samples showing dose-response results and a mutagenicity ratio >2 were considered mutagenic). All the nitrite-negative samples were nonmutagenic.

Table 2. Mutagenicity of basic extracts of some nitrite-positive cutting fluids

Samples	Dose (ml/plate)	Mutagenicity		
		Revertants/ plate	Mutagenicity ratio	Net revertants/ ml
A1	6.25	134	1.2	
	12.50	213	1.9	6
	25.00	246	2.2	
	37.50	—[a]	—	
A2	6.25	143	1.4	
	12.50	227	2.3	8
	25.00	287	2.9	
	37.50	—	—	
A3	6.25	119	1.2	
	12.50	218	2.2	8
	25.00	280	2.8	
	37.50	—	—	
B1	6.25	112	1.0	
	12.50	214	1.8	12
	25.00	392	3.4	
	37.50	—	—	
B2	6.25	296	2.5	
	12.50	514	4.4	20
	25.00	608	5.2	
	37.50	—	—	
C1	6.25	181	2.2	
	12.50	298	3.6	11
	25.00	364	4.5	
	37.50	—	—	
C2	6.25	1184	14.4	
	12.50	2280	27.8	176
	25.00	—	—	
	37.50	—	—	
G1	1.25	102	1.1	
	2.50	108	1.2	33
	6.25	296	3.0	
	12.50	—	—	

The results were obtained from preincubation assay with *S. typhimurium* TA100 strain +S9 mix and are expressed as revertants/plate and mutagenicity ratio data (revertants/plate for the sample divided by revertants/plate for solvent control). Specific mutagenicity, expressed as net revertants/ml of corresponding oil, was also calculated by linear regression analysis of the linear portion of the dose–response curves. The doses tested are expressed as volume of corresponding oil.
[a]Extract was toxic at this dose.

et al. procedure (*16*), using *S. typhimurium* TA98 and TA100 strains, with and without metabolic activation (± S9 mix).

The basic fractions were prepared and tested as follows. We adjusted the samples (150 ml) to pH 10 with NaOH, treated them with ammonium and aluminum sulfate to separate the water phase, and extracted the water phase with 3× 50-ml aliquots of ethyl ether. The ether extracts were reduced in a rotary evaporator and purified in a silica column (7 × 1 cm i.d.), using ethyl acetate (35 ml) as eluant. We dissolved the residues in dimethyl sulfoxide and tested them at different doses (up to 37.5 ml/plate of corresponding oil volume) using the preincubation modification of Ames test (*17*) with TA100 and either a post-mitochondrial liver fraction obtained from Aroclor-induced rats (+S9 mix) or alcohol dehydrogenase activation (*7,8*). This last method of activation is suitable for detecting NDELA mutagenicity.

Positive results for NDELA determined the subsequent biological monitoring program for the exposed workers. Thirteen samples (20.6%) of the 63 cutting fluids tested with the rapid method were positive for nitrites, and their quantitative determination showed a great range of nitrite content (2–19,600 mg/kg; Table 1). All the nitrite-positive samples contained very different NDELA concentrations, from traces (0.3 mg/kg) to high levels (1900 mg/kg). However, the content of nitrites was not related to NDELA content. This is probably due to different concentrations of diethanolamine, the other NDELA precursor.

The nitrite-negative samples were always nonmutagenic when tested *in toto* (TA98 and TA100 strains, ± S9) or as basic fractions (TA100 + S9 or + alcohol dehydrogenase). In contrast, 61% of the nitrite- and NDELA-positive samples (8 samples, 12.7% of the total samples) were mutagenic to TA100 +S9 in the basic extracts (Table 2). When tested *in toto*, only one sample was mutagenic to TA100 +S9. It must be pointed out that even though nitrites are mutagenic to TA100 –S9, all the samples were negative when tested using TA100 -S9 or TA98 ±S9.

Activation by alcohol dehydrogenase gave positive results only in the A3 sample, which had the highest concentration of NDELA (1900 mg/kg; Fig. 2). The negative results for the other samples could be due to the lower NDELA concentrations. Sample A3 was also the only positive sample when tested *in toto* with TA100 +S9.

The mutagenicity ratio and specific mutagenicity data revealed extremely high activity for sample C2 and high activity for samples B2 and G1. The highest doses tested (37.5 ml/plate) of all the samples were toxic to bacteria.

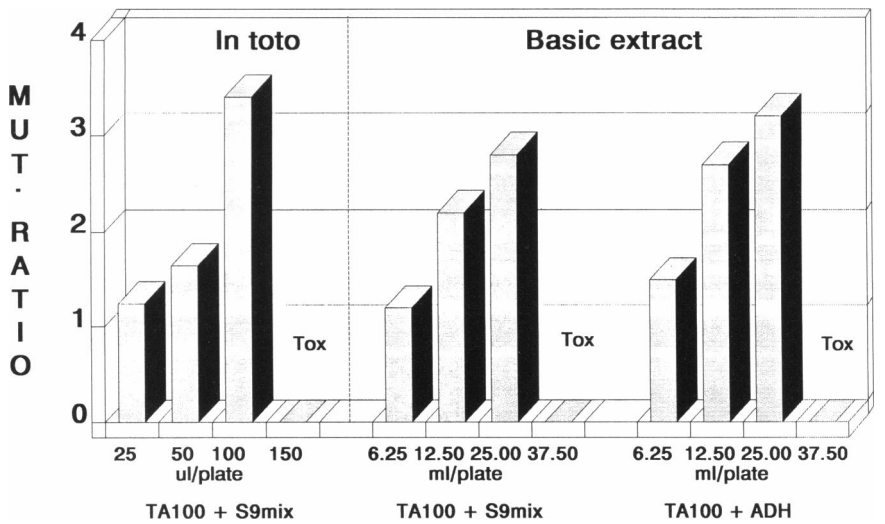

Figure 2. Dose–response curves of mutagenicity (expressed as mutagenicity ratio) found in sample A3, tested as *in toto* samples (*S. typhimurium* TA100 strain +S9 mix, plate test) and basic extracts (*S. typhimurium* TA100 strain +S9 mix or alcohol dehydrogenase preincubation test).

It should be pointed out that mutagenicity was found among the samples with the highest NDELA content (≥5 mg/kg). However, because tests with the alcohol dehydrogenase activating system were positive for only one sample, mutagenicity is probably due to other basic compounds different from NDELA. These unknown mutagens were not found in the nitrite-negative samples. Thus, unknown mutagenic substances are present in the basic extracts of these products. Identification of these compounds could lead to better estimation of the health hazards associated with exposure to cutting fluids.

In conclusion, preventive programs based on nitrite screening in the field, together with mutagenicity testing of cutting fluids, are important for identifying samples that could potentially contain NDELA and other unknown mutagens. All cutting fluids should be monitored for nitrite content before use in factories by using rapid nitrite screening tests, and the positive samples should be rejected or, as mandated in some countries, the nitrite in cutting fluids should be banned or restricted to those products that do not contain amine precursors. However, the presence of unknown potential mutagens in these fluids should be monitored using short-term mutagenicity tests both in *in toto* samples and in the basic extracts. All these measures should be carried out along with more general preventive measures in the workplace to minimize the health hazards associated with occupational exposure to cutting fluids. These results will be completed with further data, obtained from the biological monitoring program carried out among the workers of the metal factories where NDELA-positive cutting fluids are used.

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